Oxidative Stress in Hostile Environments: A Translational Omics-Based Approach to Personalized Assessment & Countermeasures Development

Thomas J. Goodwin, Ph.D.

NASA Johnson Space Center

Lead, Translational Research and Medicine

Oxidative Stress and Damage

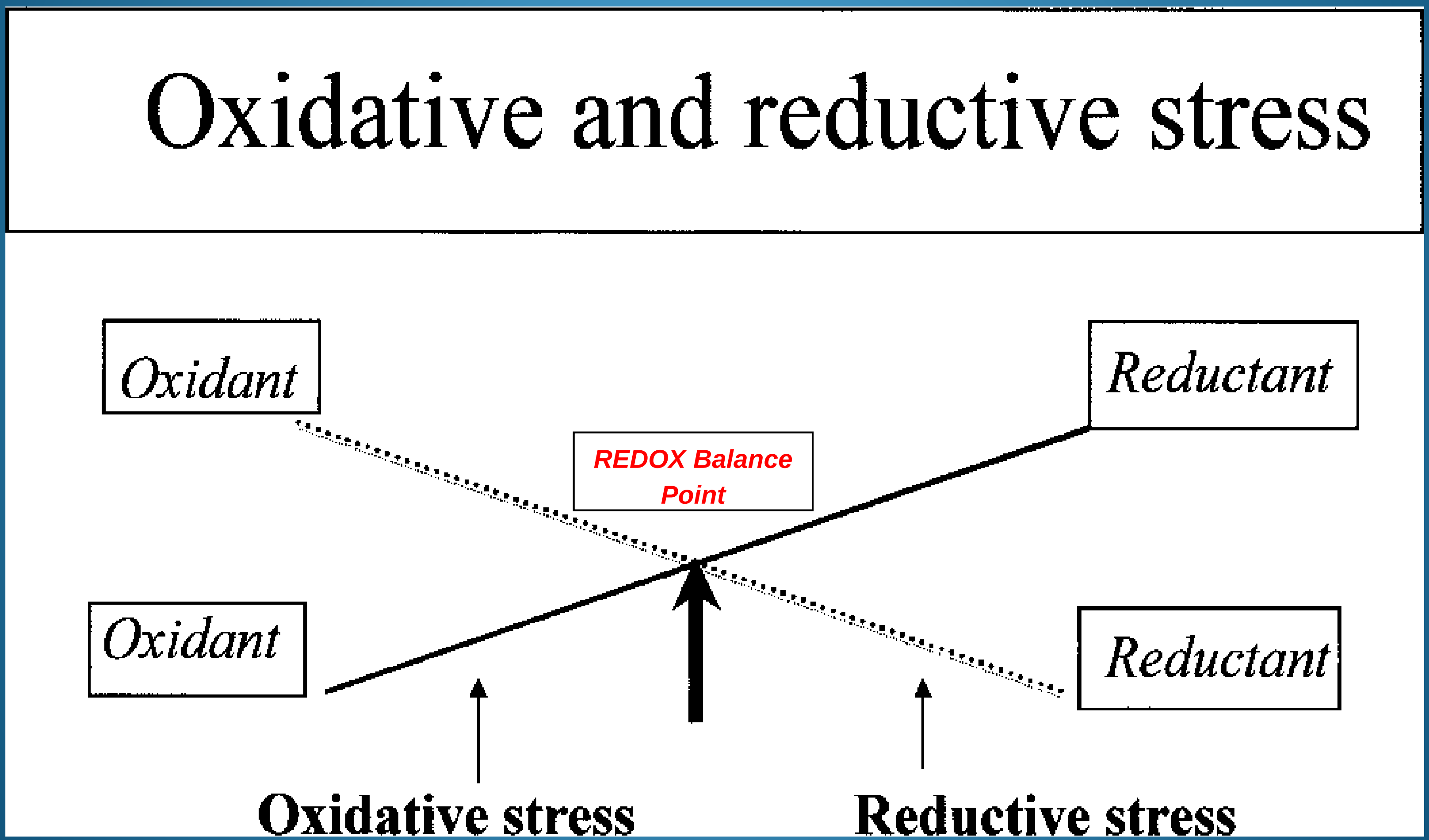
&

Michael A. Schmidt, Ph.D.

MetaboLogics, LLC

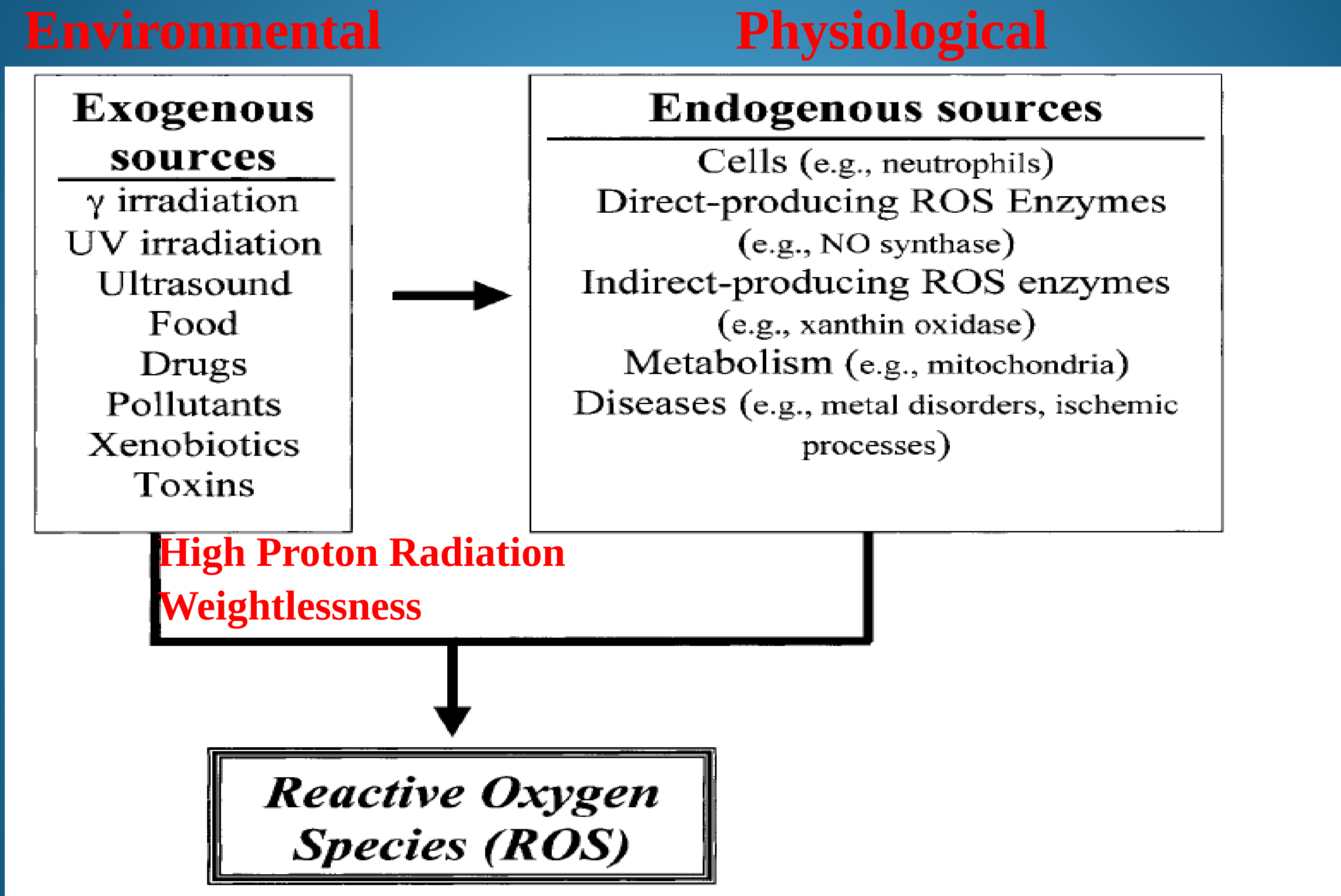
Advanced Pattern Analysis and Countermeasures Group

Oxidative Stress and Damage
A Common Denominator In Space Flight Health?



KOHEN AND NYSKA
J. TOXICOLOGIC PATHOLOGY, Vol. 30, 2002

Causal Factors/ Risks in the Production of OSaD



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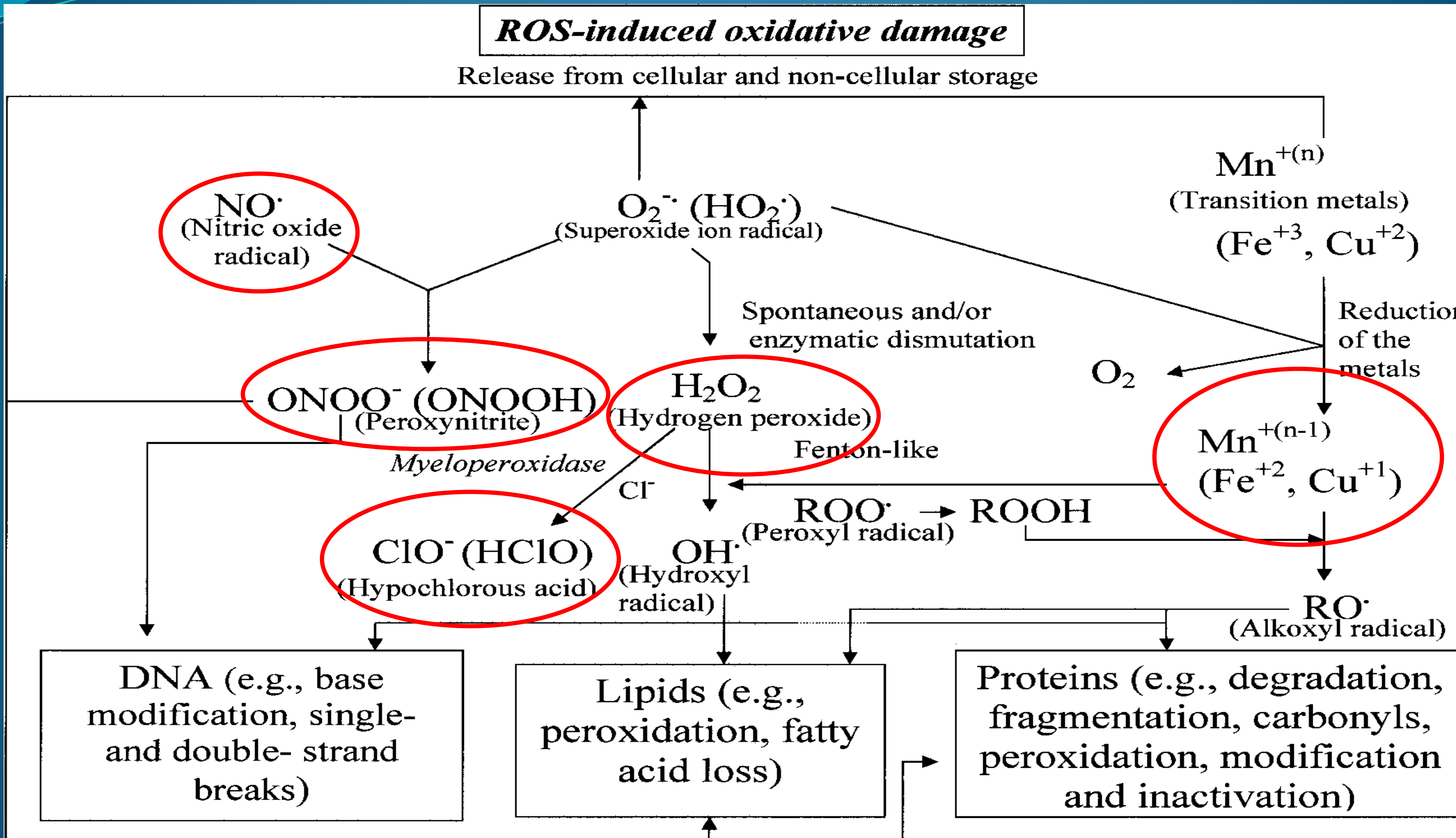
Sources of ROS



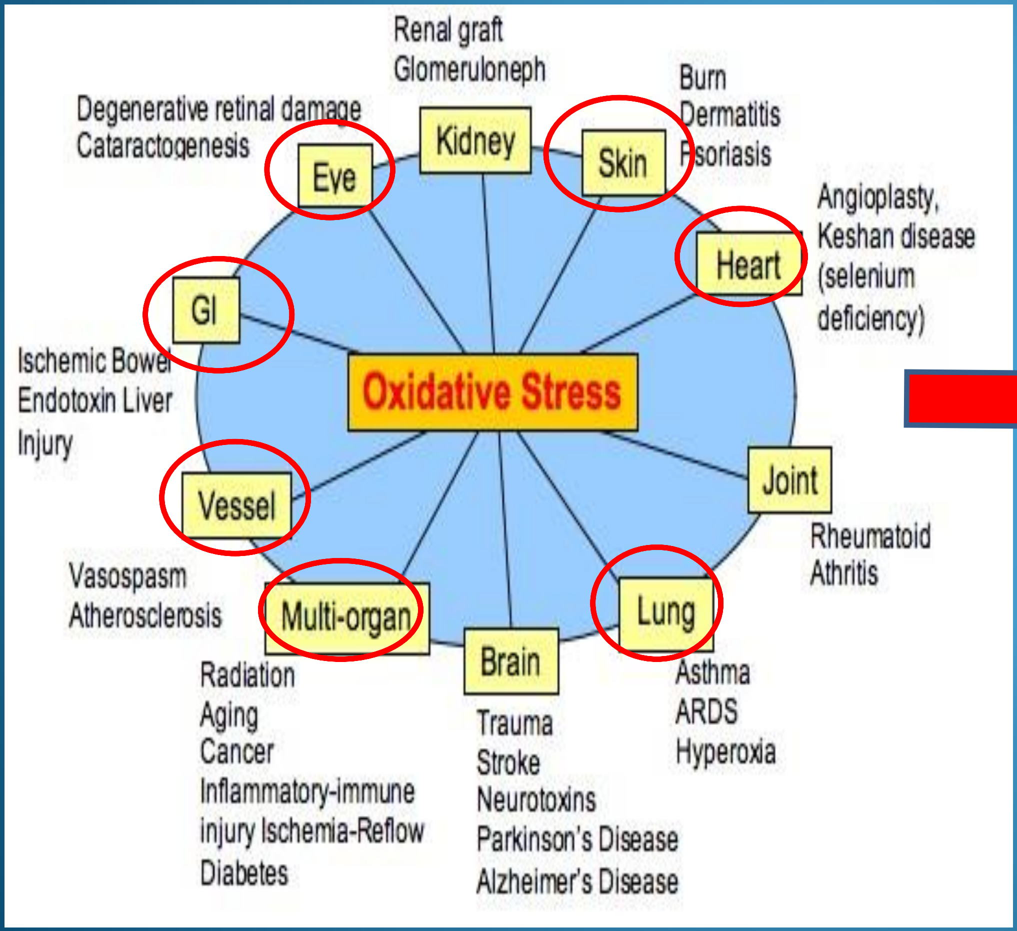
Illustrates specific free radical (ROS) species (red circles) and ions that contribute to loss of REDOX homeostasis and cellular damage, inflammation, and DNA damage. (**Adapted from Kohen R, Nyska A. Toxicol Pathol, 2002**)

Oxidative Stress and Damage Potentially Related to Space Flight Phenomena

We hypothesize that OSaD may be a contributing factor in the following areas of space flight related dysregulations:

- 1) Cardiovascular effects (Atherosclerosis)
- 2) Intracranial hypertension (VIIP)(Cephalic Fluid Shift)
- 3) Immune dysregulation and suppression
- 4) Bone loss and potential fragility
- 5) Hypoxia and Hyperoxia (DCS)
- 6) Altered one carbon metabolism linked to single nucleotide polymorphisms (SNPs)
- 7) Other relevant SNPs (e.g. HFE)

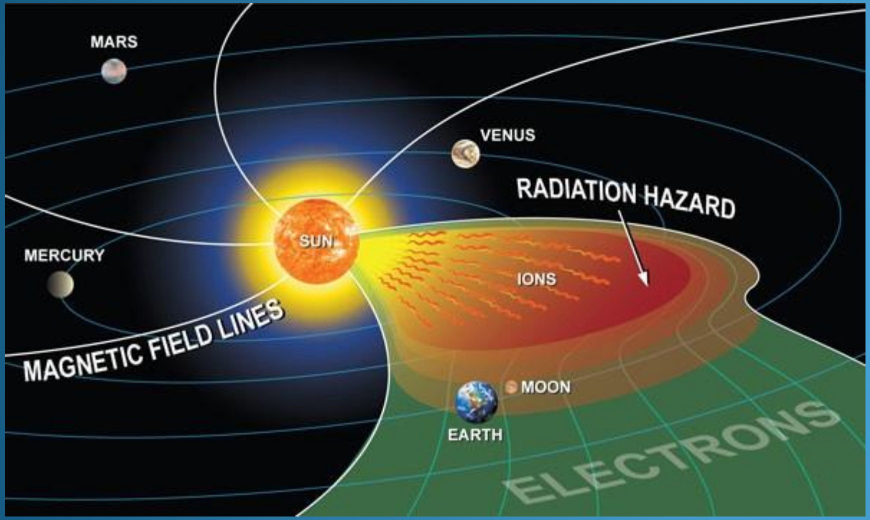
Causal Factors that Occur in 1G that also May Relate to OSaD in Space Flight

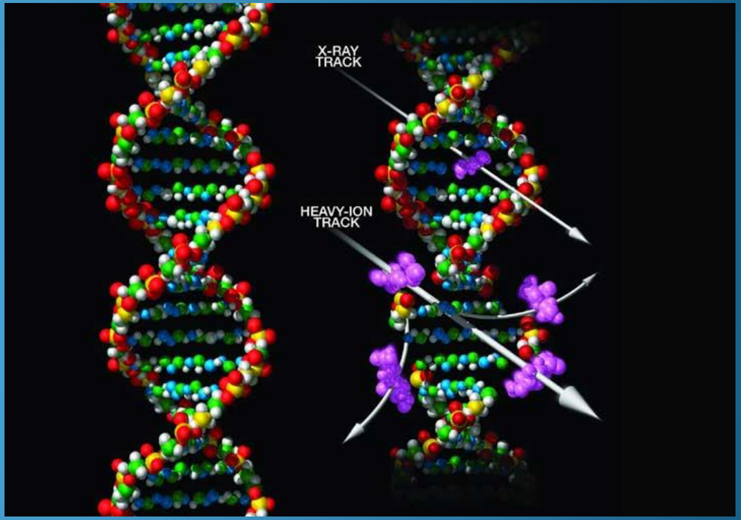


Courtesy National Institute of Standards and Technology (NIST)

Courtesy of NASA/JSC Education and Outreach

One Primary Concern in Space: Protecting Against DNA Damage





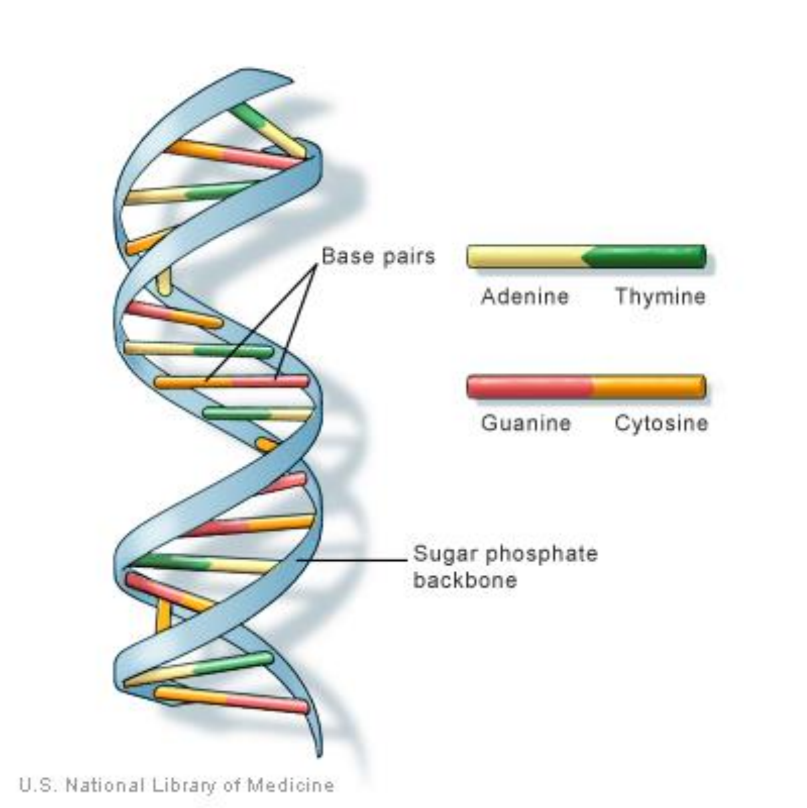
(Image Credit: NASA (assumed), via ITECS Insider)

Space Radiation

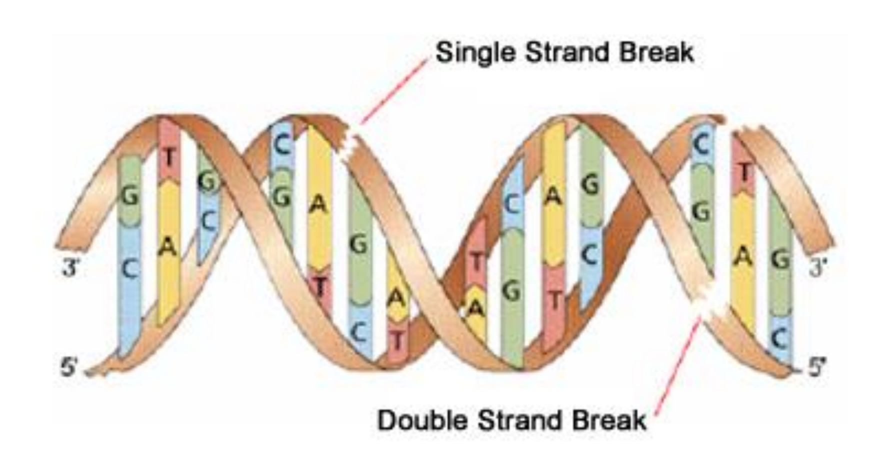
In Addition to OSaD What if…

DNA is Unstable *BEFORE* Entering Space

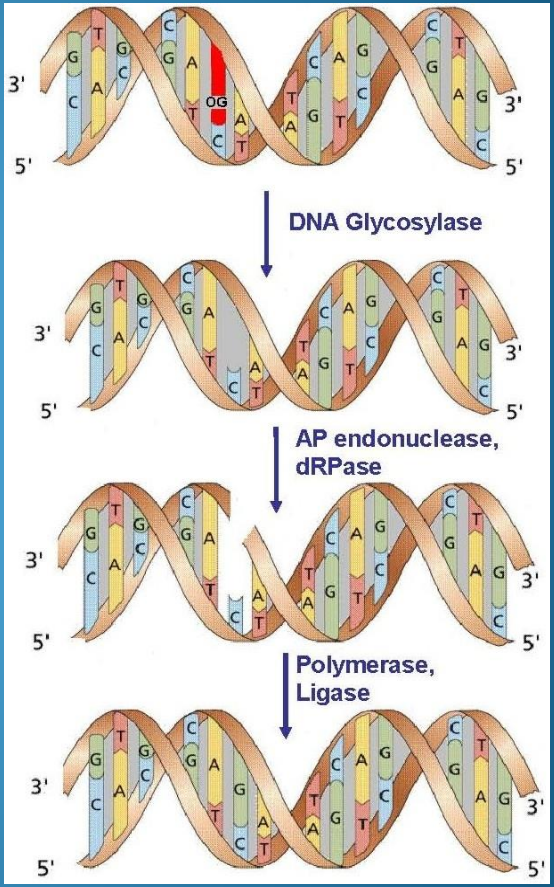
What New Countermeasures are Possible?



Two Attributes of DNA Integrity



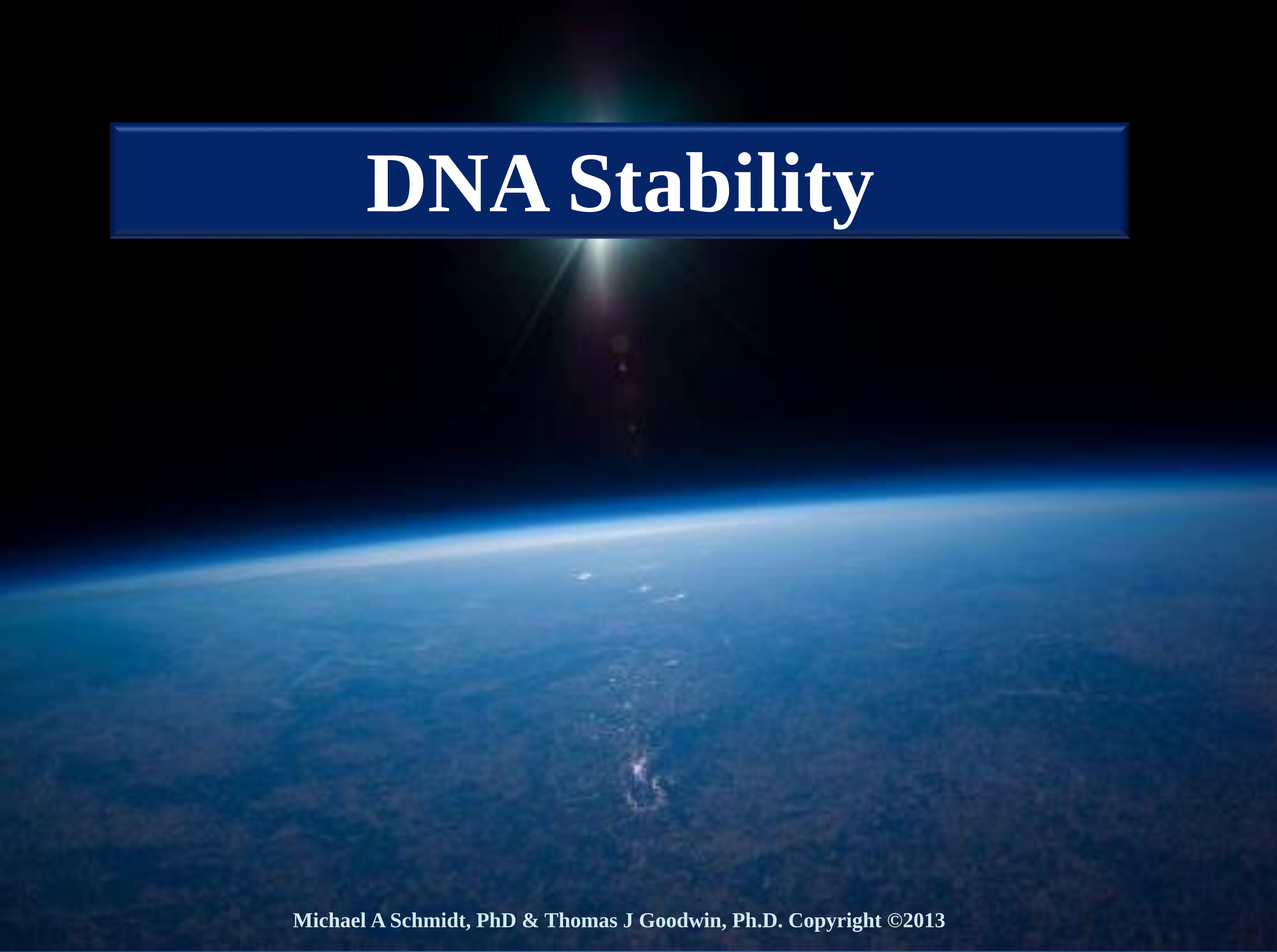
DNA Stability

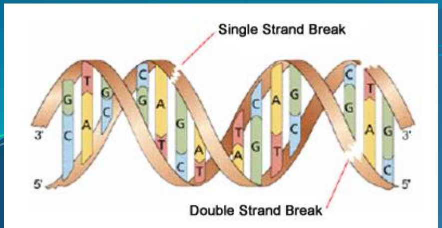


DNA Repair

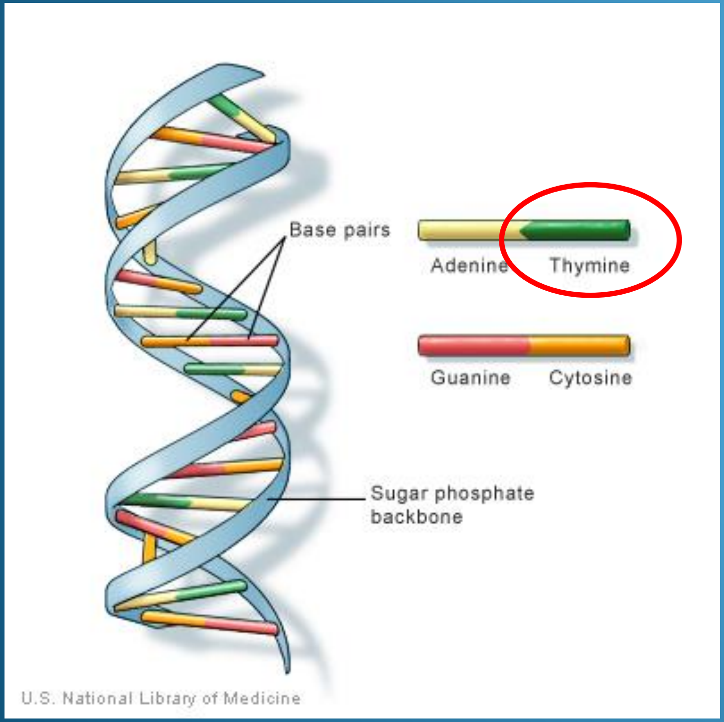
Figures adapted from Puranik, M.

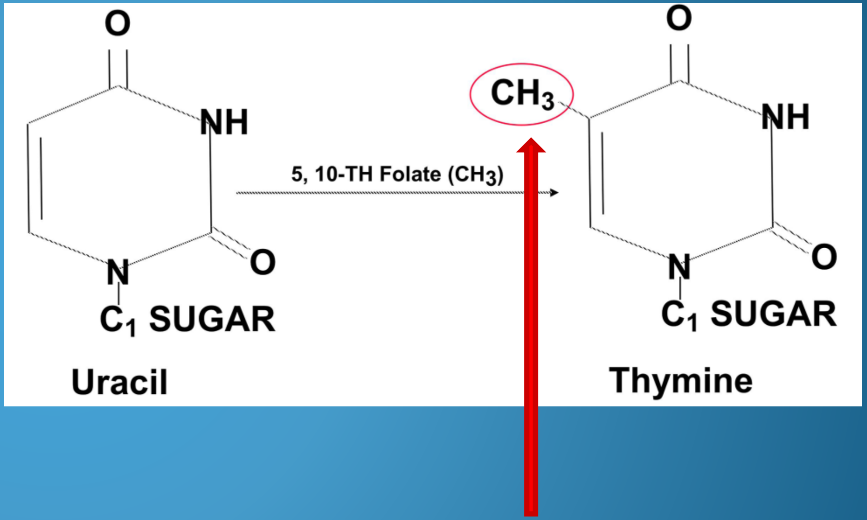
DNA Stability



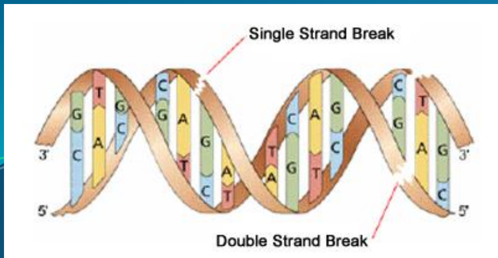


DNA Stability:
Gene & Micronutrient Influence



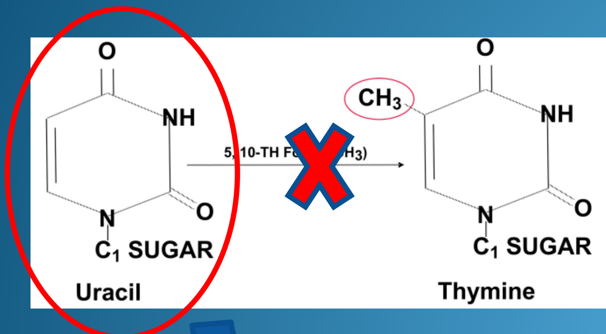


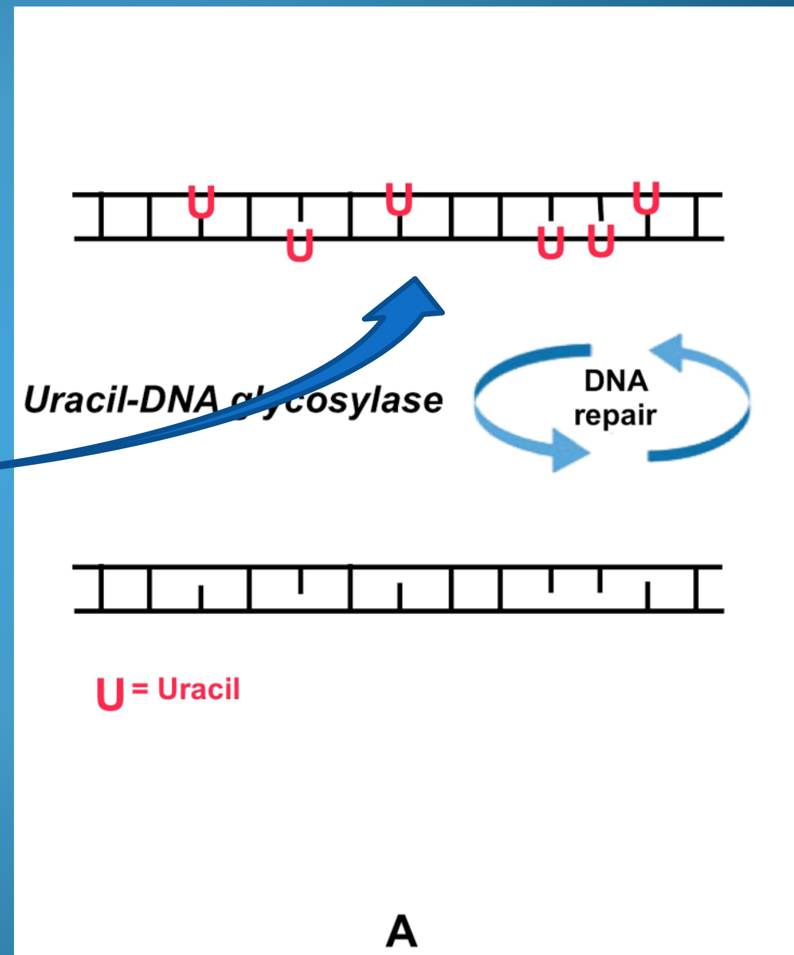
Genetics (MTHFR, MTR, etc.)
Micronutrients (folate, B12, etc.)



DNA Stability:

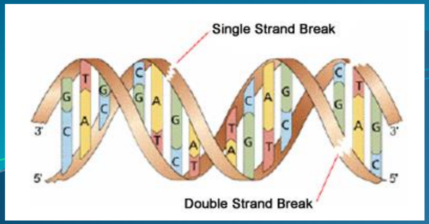
Uracil Misincorporated into DNA Strand





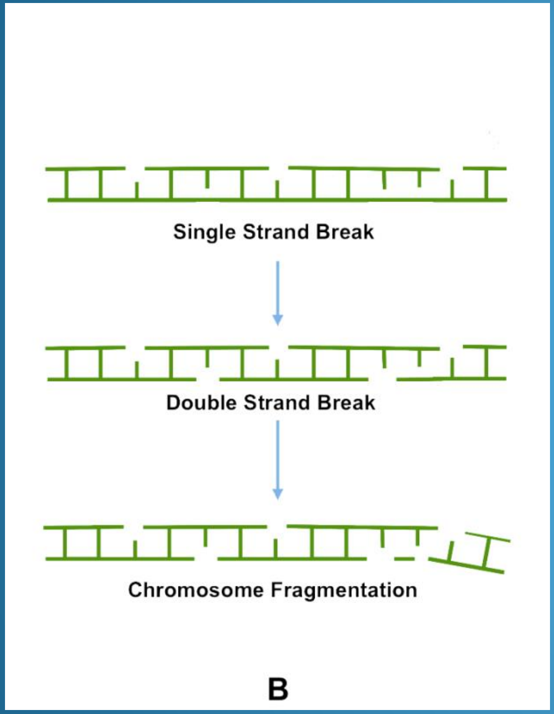
Genetics (MTHFR, MTR, etc.)
Micronutrients (folate, B12, etc.)

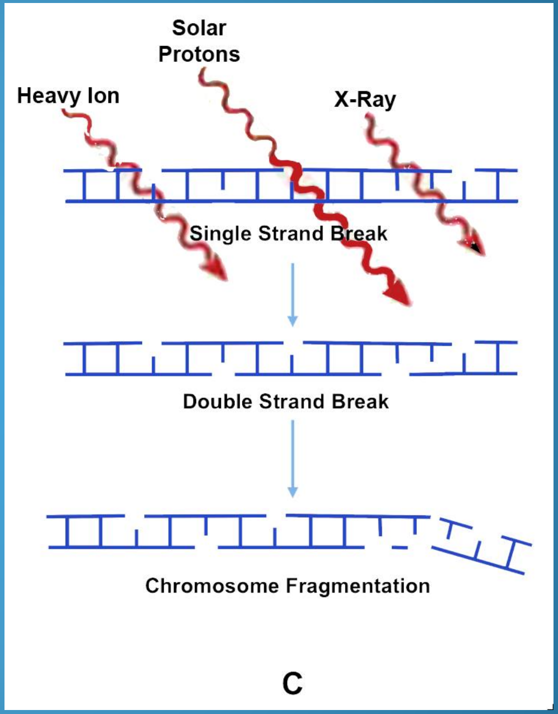
From: Schmidt, MA, Goodwin, TJ. (2013) Personalized Medicine in Human Space Flight. *Metabolomics*.



DNA Stability:

If poor methyl status, uracil accumulates

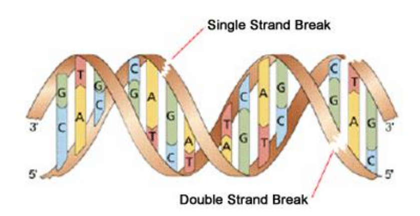




Uracil > unstable DNA-Mutational Event 1

Radiation strikes unstable DNA-Mutational Event 2

From: Schmidt, MA, Goodwin, TJ. (2013) Personalized Medicine in Human Space Flight. *Metabolomics*.

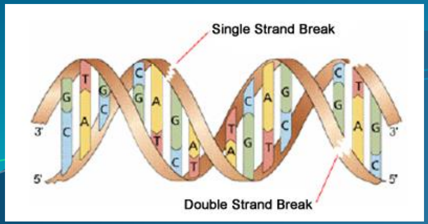


DNA Stability:
High Uracil, Folate Corrects

- Blount et al.: Uracil levels were found to be 70 times higher in subjects whose serum folate < 4 ng/ml (as high as 4M uracil residues/cell).
- After three days supplementation with 5 mg folic acid, uracil levels were rapidly reduced. (Roughly 12x RDI of 400mcg/d)
  - **RDI=Recommended Daily Intake for Healthy Individuals**
- Are Healthy individuals still health in Space?

Blount, B. C., et al. (1997). Proc Natl Acad Sci U S A, 94, 3290-3295.

From: Schmidt, MA, Goodwin, TJ. (2013) Personalized Medicine in Human Space Flight. *Metabolomics*.



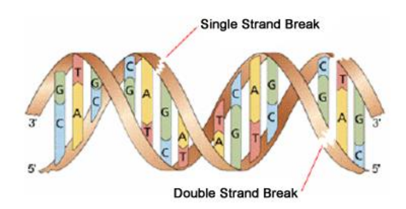
DNA Stability:

If poor methyl status, damage similar to and possibly compounded by elevated radiation

- Chromosomal damage caused by reducing folate concentration from 120 to 12 nmol/L
- Equivalent to that induced by an acute exposure to 0.2 Gy of low linear-energy-transfer ionizing radiation (*e.g.*, X-rays)
- A dose of radiation that is 10 times greater than the annual allowed safety limit of exposure for the general population

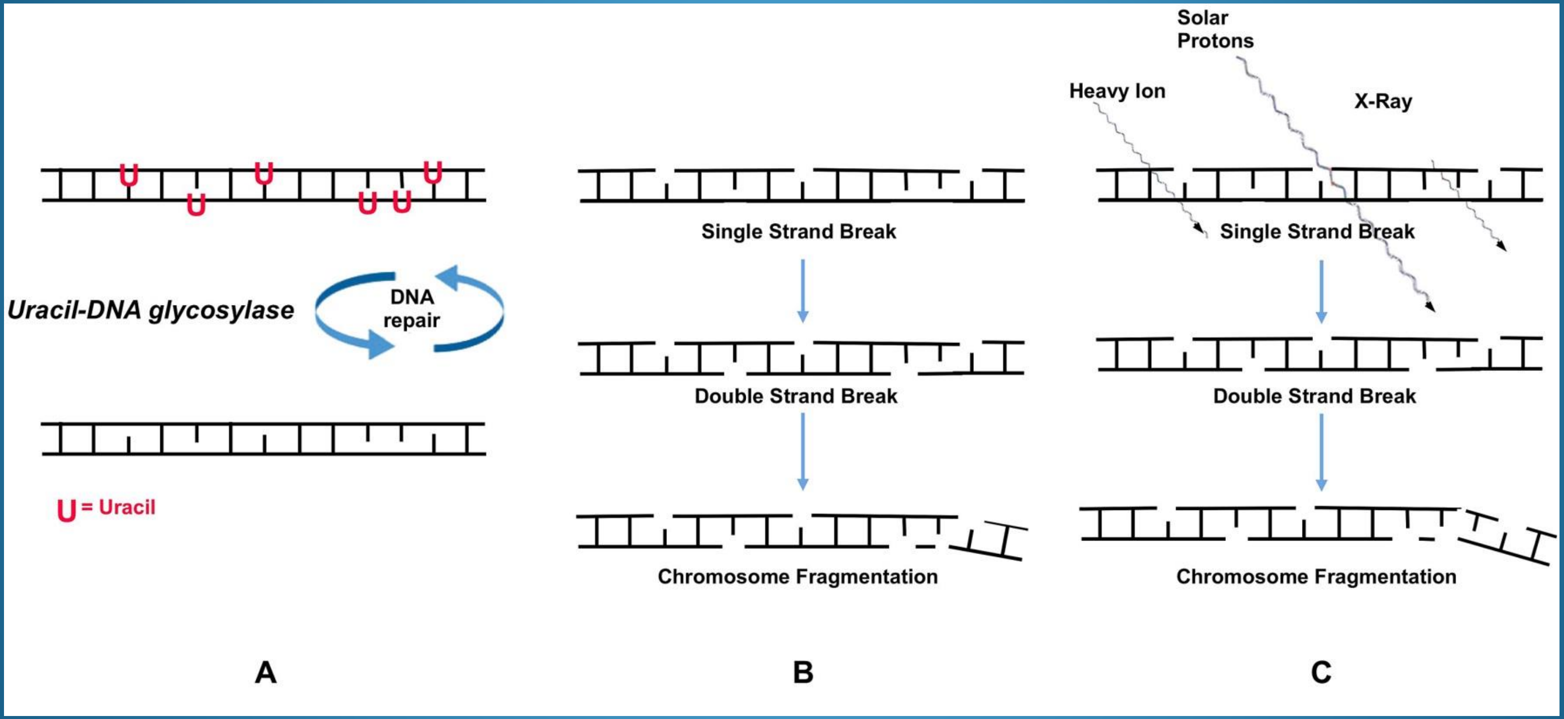
Fenech, M. (2010) Am J Clin Nutr, 91, 1438S-1454S.
Fenech, M. (2012) Mutation Research, 733:21-33.

From: Schmidt, MA, Goodwin, TJ. (2013) Personalized Medicine in Human Space Flight. *Metabolomics*. *In Press*



Gene & Micronutrient Variants Reduce DNA Stability

Schmidt, MA, Goodwin, TJ. (2013) Personalized Medicine in Human Space Flight. *J. Metabolomics*.



Courtemanche, C., Huang, A. C., Elson-Schwab, I., et al. (2004). Folate deficiency and ionizing radiation cause DNA breaks in primary human lymphocytes: a comparison. FASEB J, 18, 209-211.
Fenech, M. (2012) Folate (vitamin B9) and vitamin B12 and their function in the maintenance of nuclear and mitochondrial genome integrity. Mutation Research, 733:21-33.

Are One Carbon Deficits Common in Astronauts?

ISS Study: Those with (OC^+; n=5) and without (OC^-;n=15) ophthalmic changes

Factor	OC^+	Finding
Metabolite	Homocysteine Methylmalonic Acid Cystathionine	Elevated Elevated Elevated
Essential Input (Nutrient)	Folic Acid B12 B6	Low in 4/5 OC^+ Low Low (not signif)
Genotype (MTHFR, MTRR, etc)	Unknown	Unknown

Smith, SM, et al. Vision Changes after Spaceflight Are Related to Alterations in Folate– and Vitamin B-12–Dependent One-Carbon Metabolism. J. Nutr. doi: 10.3945/jn.111.154245.

How Common Are One Carbon Variants?

Widespread in Population

	CC (n)	CT (n)	TT (n)
MTHFR *C677T*			
1298AA	1492	2217	783
1298AC	2469	1848	16
1298CC	1246	43	0

86% of 10,601 people possessed at least one mutant MTHFR allele (MTHFR *C677T* and *A*1298*C*). Fully 17.4% were heterozygous for both MTHFR *C677T* and *A*1298*C*.

Fredriksen, A, Meyer, K, Ueland, PM, et al. Large-scale population-based metabolic phenotyping of thirteen genetic polymorphisms related to one-carbon metabolism. Human Mutation 2007;28(9):856-65.

Folate, B12: Keeping DNA Stable

Schmidt, MA, Goodwin, TJ (2013) Personalized Medicine in Human Space Flight. *Metabolomics* In Press

	Prevent Anemia	Minimize DNA Damage
Plasma Folate (ng/ml)	2.2	21 (7.3-53.0)
RBC Folate (ng/ml)	132	464 (313-600)

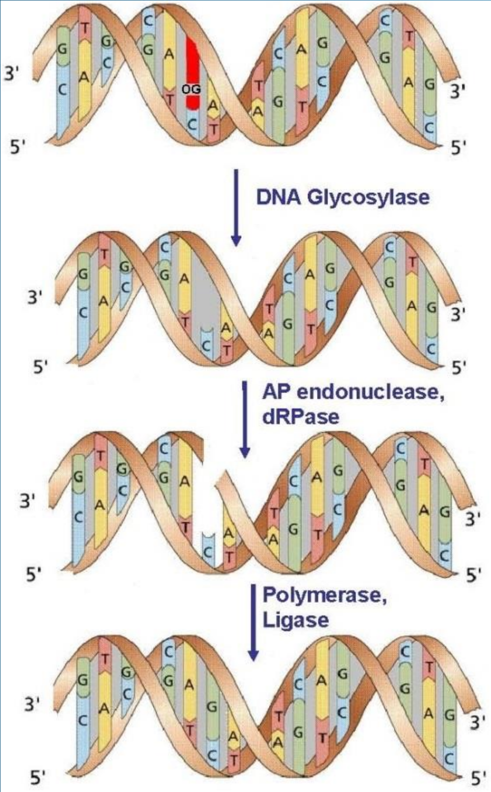
	Prevent Anemia	Minimize DNA Damage
Plasma B12 (pmol/L)	150	400

Beck, S, Olek, A. The Epigenome, 2003

Fenech et al. Carcinogenesis, 1998;19:1163-71; Fenech 2012

DNA Repair and Energy Production

DNA Repair: Mg Dependence



Figures adapted from Puranik, M.

- Mg is important for maintaining the integrity of DNA

- Mg cations (Mg^{++}) bind to DNA and reduce the negative charge density, thereby stabilizing the structure of DNA

- Mg is cofactor for the enzymes:
  - nucleotide excision repair
  - base excision repair
  - mismatch repair (MMR)

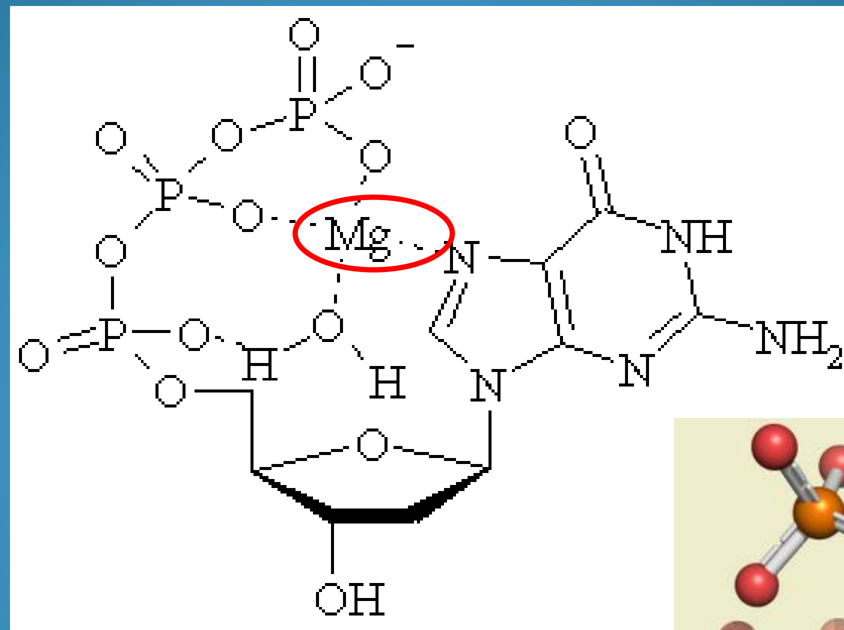
- MMR activity lowers the mutation frequency in the genome by 2–3 orders of magnitude
- **DNA repair REQUIRES ATP**

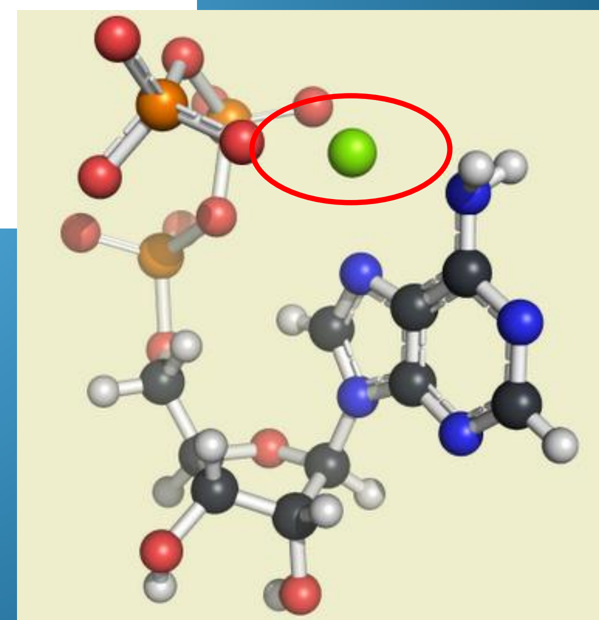
-Anastassopoulou, J. et al. (2002) Magnesium-DNA interaction and the possible relation of magnesium to carcinogenesis. Irradiation and free radicals. Crit. Rev. Oncol. Hematol., 42, 79–91.
-Hartwig, A. (2001) Role of magnesium in genomic stability. Mutat. Res., 475, 113–121.

12
Mg
Magnesium
24.31

Magnesium-ATP Complex

Magnesium-ATP complex *__is the sole biologically active form of ATP__*



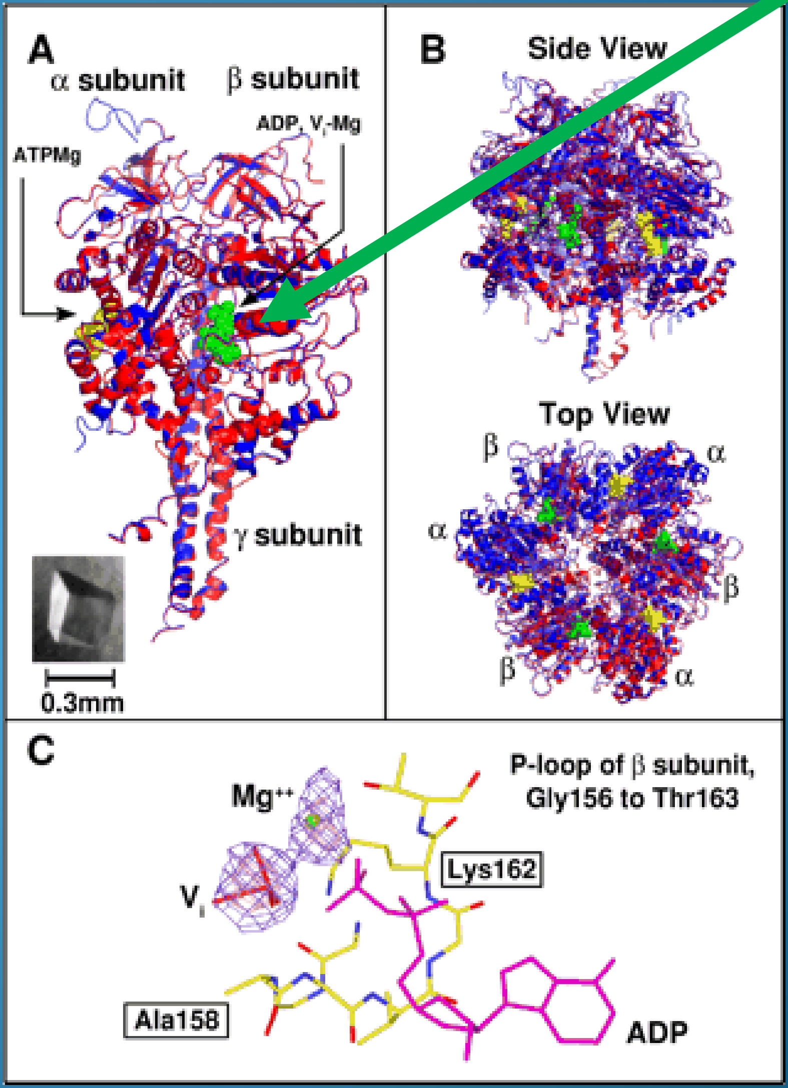


All ATP reactions involve Mg

12
Mg
Magnesium
24.31

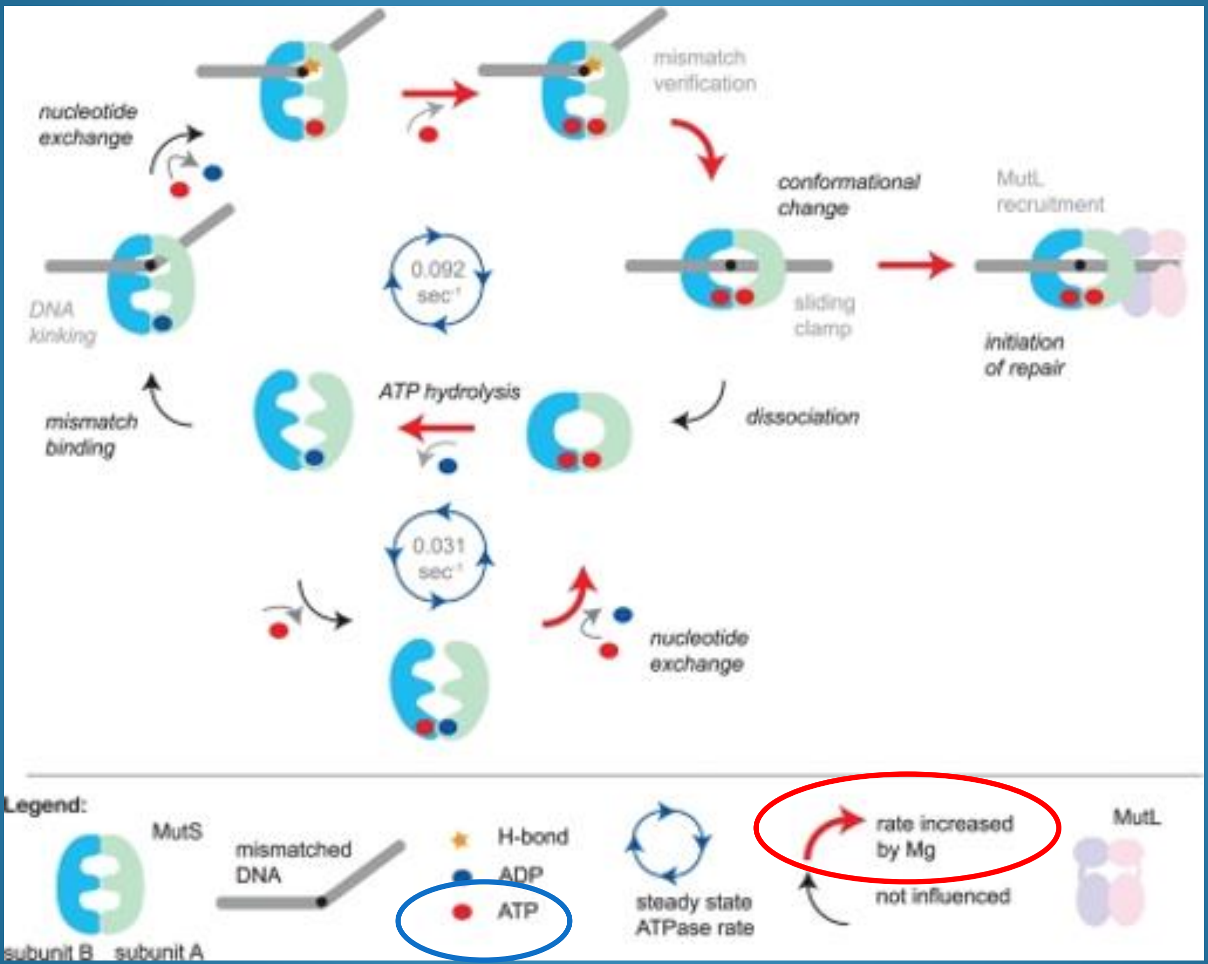
Magnesium-ATP Synthase

ATP Synthase also requires magnesium



JBC May 12, 2006 vol. 281 no. 19 13777-13783

Magnesium Controls DNA Mismatch Repair Switch: Needs ATP



Lebbink JH, et al. Magnesium Coordination Controls the Molecular Switch Function of DNA Mismatch Repair Protein MutS* J. Biol. Chem. 2010:285(17);13131-41.

Magnesium and Spaceflight

- After 6 months in space:
  - Loss of Mg reservoirs
  - 35% loss in some leg muscles
  - ~1-2% average loss per month in bone
  - Decreased plasma volume ~15-20%
  - Loss precipitated by reduced gravity, Cephalic fluid shifts, and stress related reactions to confinement

- Smith, S. M., et al. (2005). J Bone Miner Res, 20, 208-218.
- Smith, S. M., et al (2005). J Nutr, 135, 437-443.
- Fitts RH, et al. J Physiol 2010;588(Pt 18):3567–3592

Magnesium and Spaceflight

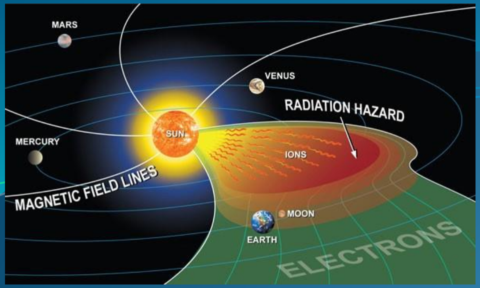
- <u>Urinary Mg levels:</u>
- 44% lower after landing than before launch (*P* < 0.001)
- 55% of ISS crew members had Mg concentrations lower than the low end of the clinical range (3.0 mmol/d)

- <u>Question:</u> Are we flying astronauts with diminished DNA repair capacity Unknowingly?

- Smith, S. M., et al. (2005). J Bone Miner Res, 20, 208-218.
- Smith, S. M., et al (2005). J Nutr, 135, 437-443.
- Fitts RH, et al. J Physiol 2010;588(Pt 18):3567–3592

Convergent Influences in Space Medicine

Unstable DNA + Altered DNA Repair + Radiation



(Image Credit: NASA (assumed), via ITECS Insider)

Convergent Influences

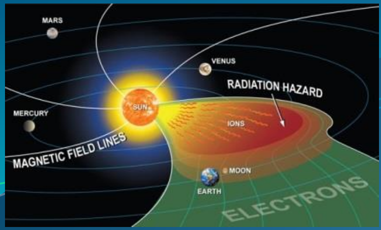
Opportunity for Countermeasures

Radiation Hostile Environment

Unstable DNA
Many Variants

Altered DNA Repair
Many Variants

Astronaut Safety & Performance



Convergent Influences

Opportunity for Countermeasures

Unstable DNA
Many Variants

Radiation

Altered DNA Repair
Many Variants

Astronaut Safety & Performance

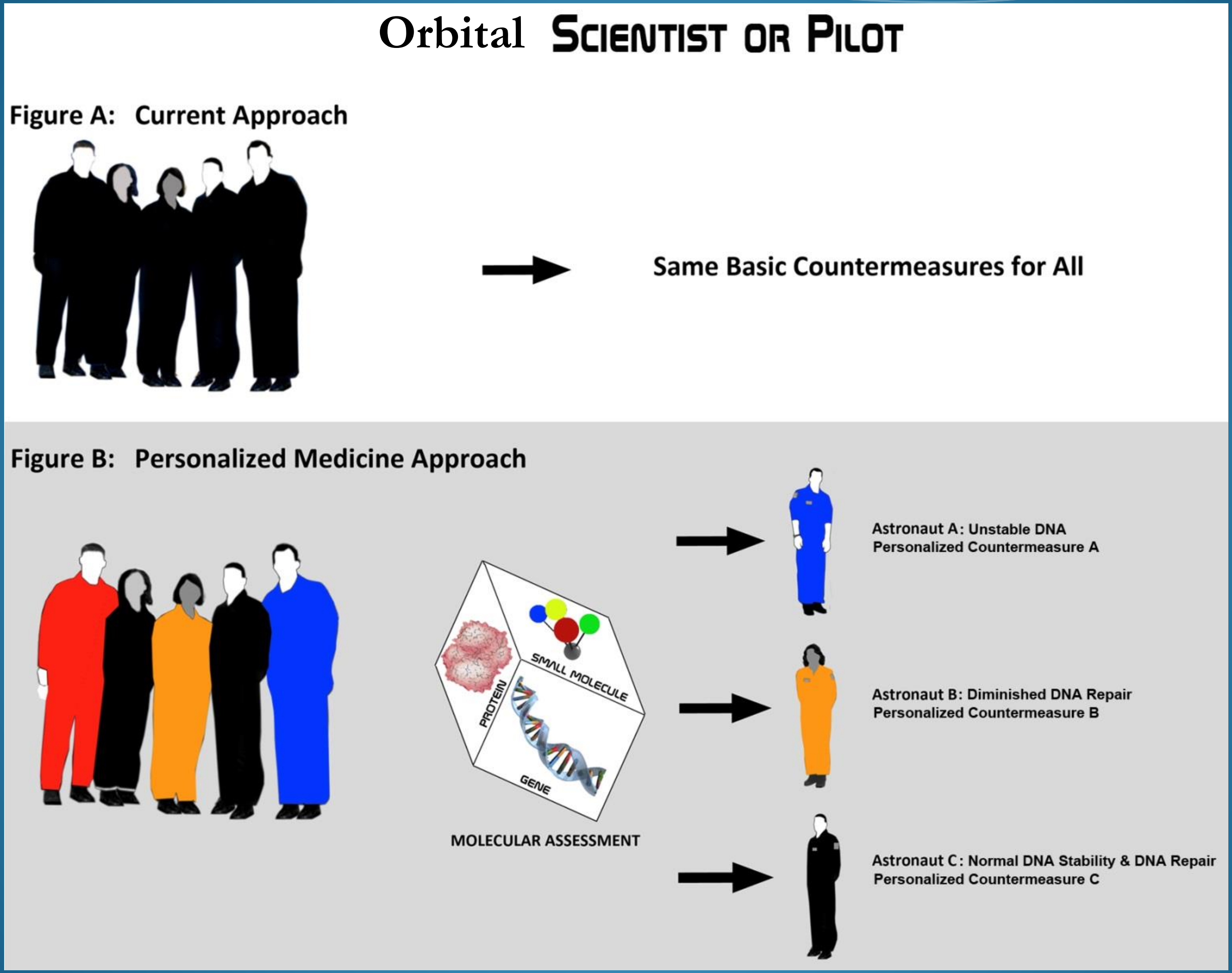
<u>SNPs: One Carbon</u>

- MTHFR (C677T, A1298C)
- MTR (A2756G)
- MTRR (A66G)
- BHMT (G742A)
- CBS (C699T)
- TCN2 (C776G)

<u>Mg: DNA Repair</u>

- DNA glycosylases
- AP endonucleases
- End processing enzymes
- DNA polymerases
- Flap endonuclease
- DNA ligase
- Mismatch Repair
- Base excision repair
- ATP synthase (All)
- ATP stabilization (All)

Using Omics to Drive Research & Personalize Care



From: Schmidt, MA, Goodwin, TJ. (2013) Personalized Medicine in Human Space Flight: Using Omics Based Assessment to Develop Individualized Countermeasures that Enhance Astronaut Safety and Performance. *J. Metabolomics.*